SUPPLEMENTARY MATERIALS AND METHODS

2D-WESTERN BLOT ANALYSIS

For 2D-Western blotting, 100 µg of proteins from MT fractions were subjected to isoelectrofocalisation using linear pH4–7 strips (GE-Healthcare) prior to second dimension electrophoresis, as described previously [3]. SDS-PAGE, transfer and immunodetection were performed as described in the experimental procedure section.
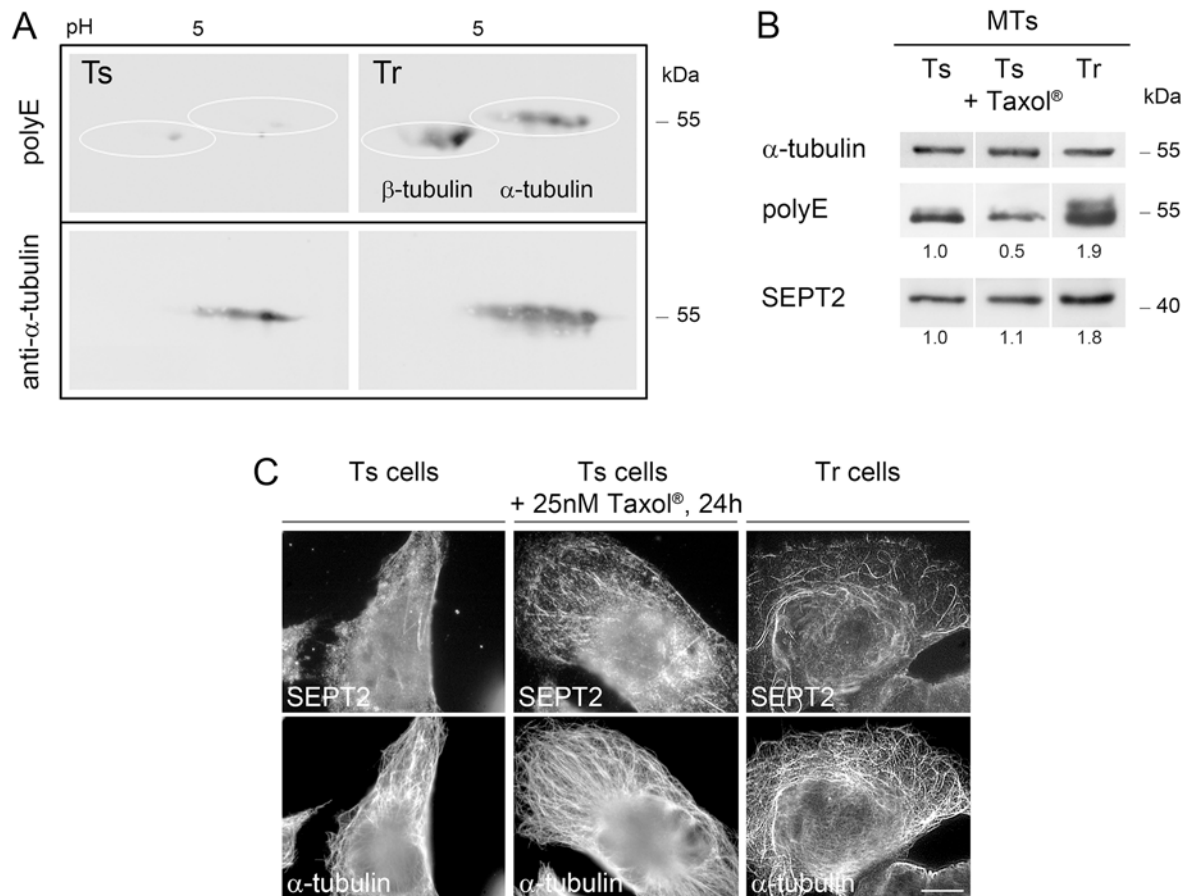
RT-PCR ANALYSIS

Total RNA was isolated using Extract-ALL phenol-guanidine reagent (Eurobio) and chloroform, precipitated with isopropanol, and was reverse transcribed with SuperScriptIII (Life Technologies) according to the manufacturer's instructions. Real-time PCR was performed with SYBR Green (Applied Biosystems) detection using primers specific for *SEPT9_v1* (forward CGGAGGCACCATGAAGAAGT, reverse GGGCCACTGGAGTCACCAA), *SEPT9_v4** (forward GGCAGGAGTGTCATCTTTTC, reverse GCGTGCCTCCTGGACA), *TTLL1* (forward GGTGCTCTGTCAGTGCCTATG, reverse TGCACACTCATCCAGTAAAAATTC), *TTL* (forward GAGGCTTCAGAGCTTCTCGA, reverse CCAGCTTCGGATGTCAAACT) and *CCP1* (forward GCTGCCCCTTGGTGACTATA, reverse TTCTCCAGGATGTACCCGAG).
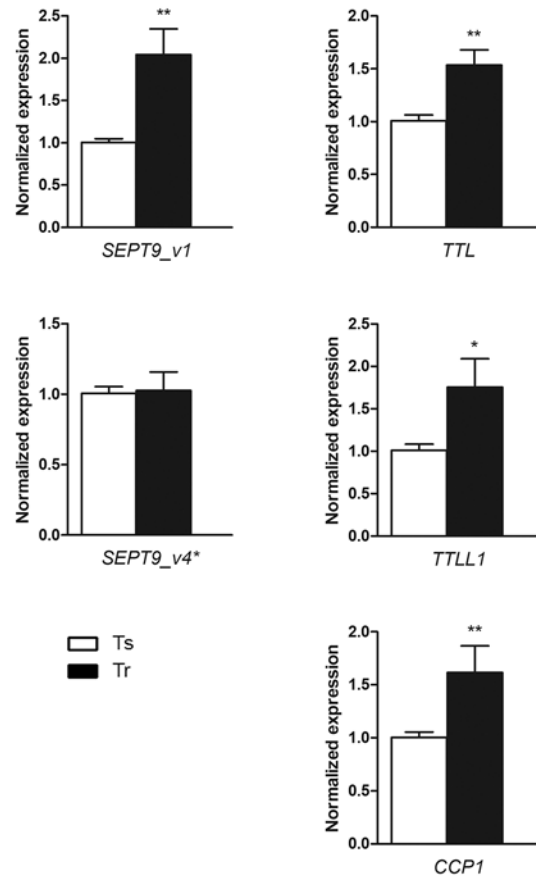
Relative gene expression is the ratio of the mRNA content of target genes to those of human *GAPDH* (forward CAGCCTCAAGATCATCAGCA, reverse TGTGGTCATGAGTCCTTCCA), 18S (forward TGAGAAACGGCTACCACATC, reverse TTACAGGGCCTCGAAAGAGT) and TBP-H2 (forward GAGAGCCACGAACCACGG, reverse ACATCACAGCTCCCCACCAT) house-keeping genes, according to the ΔΔCt method [84]. Reverse and forward oligonucleotide primers, specific to the chosen candidate and housekeeping genes, were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

REFERENCES

84. Livak KJ. Analysis of relative gene expression data using real-time quantitative PCR and the 2) Method. Methods. 2001; 25:402–408.

85. Spiliotis ET, Kinoshita M, Nelson WJ. A mitotic septin scaffold required for Mammalian chromosome congression and segregation. Science. 2005; 307:1781–1785.

86. Montagna C, Lyu MS, Hunter K, Lukes L, Lowther W, Reppert T, Hissong B, Weaver Z, Ried T. The Septin 9 (MSF) gene is amplified and overexpressed in mouse mammary gland adenocarcinomas and human breast cancer cell lines. Cancer Res. 2003; 63:2179–2187.

Supplementary Figure S1: A. Both α- and β-tubulin display long polyglutamylated chains in Tr cells. Levels of polyglutamylation (polyE signal) of α- and β-tubulin were analyzed by 2D-Western blotting on MT fractions of Ts and Tr cells. **B.** MTs of Ts cells treated with 25 nM Taxol® for 24 h do not exhibit a higher level of long chain polyglutamylated tubulin (polyE signal) and septin recruitment than Ts cells. **C.** Acute Taxol® treatment of Ts cells (25 nM Taxol®, 24 h) does not relocalize septins to MTs. Scale bar = 10 μm**.**

Supplementary Figure S2: Transcript expression of septins and polyglutamylation enzymes were evaluated by qPCR analysis in Ts and Tr cells. Histograms represent normalized transcripts expression.

Supplementary Table S1: siRNA used to deplete cells of septins, tubulin modification enzymes and +TIPs

siRNAs	Suppliers	Sequences
SEPT2 1	Sigma CUSTOM oligos [85]	sense 5′-AAGGUGAAUAUUGUGCCUGUC [dT][dT]-3′ antisense 5′-GACAGGCACAAUAUUCACCUU [dT][dT]-3′
SEPT2 2	Sigma MISSION SASI_Hs02_00305472	sense 5′-GAAUGAGGACAUGAAUAAA [dT][dT]-3′ antisense 5′-UUUAUUCAUGUCCUCAUUC [dT][dT]-3′
SEPT9 1	Sigma CUSTOM oligos [86]	sense 5′-GUCCACUUUAAUCAAUACC [dT][dT]-3′ antisense 5′-GGUAUUGAUUAAAGUGGAC [dT][dT]-3′
SEPT9 2	Sigma MISSION SASI_Hs02_00325891	sense 5′-GUGUGCAGAUCAUCCGUCU [dT][dT]-3′ antisense 5′-AGACGGAUGAUCUGCACAC [dT][dT]-3′
SEPT11	Sigma CUSTOM oligos [65]	sense 5′-CAAGAGGAAUUGAAGAUUAAA [dT][dT]-3′ antisense 5′-UUUAAUCUUCAAUUCCUCUUG [dT][dT]-3′
TTL 1	Sigma MISSION SASI_Rn02_00198137	sense 5′-CCAACUAAGAGGUCAUUAA [dT][dT]-3′ antisense 5′-UUAAUGACCUCUUAGUUGG [dT][dT]-3′
TTL 2	Sigma MISSION SASI_Hs01_00198137	sense 5′-GAGUUCAAUCAGUACCUAA [dT][dT]-3′ antisense 5′-UUAGGUACUGAUUGAACUC-3′
TTL 3	Santa Cruz (sc-94871)	
TTLL1 1	Sigma MISSION SASI_Hs01_00245872	sense 5′-GACAUCAUCAUCGACGACA [dT][dT]-3′ antisense 5′-UGUCGUCGAUGAUGAUGUC-3′
TTLL1 2	Santa Cruz (sc76772)	
TTLL5	Sigma MISSION SASI_Hs01_00168438	sense 5′-GAAACUGUCUCGUCCCUCU [dT][dT]-3′ antisense 5′-UGUCGUCGAUGAUGAUGUC-3′
TTLL11	Sigma MISSION SASI_Hs01_00141865	sense 5′-CAUCUACUGGCAUGGAGUU [dT][dT]-3′ antisense 5′-AACUCCAUGCCAGUAGAU-3′
CLIP-170	Sigma CUSTOM oligos	sense 5′-GCACAGCUCUGAAGACACC [dT][dT]-3′ antisense 5′-GGUGUCUUCAGAGCUGUGC [dT][dT]-3′
MCAK	Santa Cruz (sc-105596)	